Supplemental information for

Evaluation of conventional and alternative monitoring methods for a recreational marine beach with non-point source of fecal contamination

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Water Sampling (Sinigalliano et al. 2010)

Study subjects were adults who reported regular bathing in South Florida marine waters who were randomly assigned to either (exposed) bather group or (unexposed) non-bather group. The bathers were assigned to the bathing station where staff members supervised the exposure activity of each bather, including the time, location, unusual activities, and duration each individual bather spent in the water. Bathers were required to spend 15 minutes in knee-deep water (due to the relative shallowness of the study site), and to immerse their head three times completely under water. Using ropes, a 30-40-meter stretch of beach was subdivided into 5 meter intervals forming 6-8 bathing exposure zones with exposure of any individual bather restricted to their own individual 5 -meter-wide swim zone. Each subject was instructed to take their own water sample at 5 minute intervals near the surface before their head immersion, as well as provided with an appropriate individual water sampling container. Staff members instructed participants to thoroughly rinse the collection container before filling completely with the marine water, as well as the avoidance of microbial contamination of the collection container by the participant. When the subjects left the water, they gave their individual water samples to the environmental research study staff for microbial analysis processing (described below). No bather was allowed to enter the water more than once during the actual study exposure. The participants in the randomized non-bather group were restricted to sitting on chairs on plastic sheeting in a covered roped-off area distant from water and sand exposure for 15 minutes (Fleisher et al. (in press) and Sinigalliano et al. 2010).

Additional Details for Laboratory Microbiological Analysis of Samples

Fifty milliliter sub-samples taken from the individual 5-liter water samples were aseptically filtered onto 0.45 um pore-sized Whatman cellulose nitrate filters and assayed for culturable enterococci by the standard membrane filtration (MF) method as recommended by the US EPA (EPA Method 1600 – US EPA, 1997, 2000). Culturable enterococci were also enumerated by the Chromogenic Substrate (CS) method (EnteroLert™ with the QuantiTray-2000™, IDEXX, Westbrooke, Maine), using sample dilutions of 1:10 and 1:20 and following the manufacturer's recommendations for marine waters (Sinigalliano et al. 2010).

S. aureus was isolated from 50 mL water samples using a standard membrane filter (MF) followed by growth on selective media, Baird Parker agar (Becton, Dickinson and Company, Sparks, MD) with Egg Yolk (EY) Tellurite Enrichment (Becton, Dickinson and Company, Sparks, MD). Filter membranes were incubated aerobically at 37 °C for a minimum of 24h. After incubation, colonies found to be black, shiny, convex, 2-5mm in diameter, and surrounded by clear zones (BP) were considered presumptive *S. aureus*, and subjected to confirmatory tests. Presumptive positive isolates were transferred to Mannitol Salt agar for the determination of mannitol fermentation, and incubated aerobically at 37 °C for 16-24h. Mannitol-fermenting isolates were transferred to Trypticase Soy Agar with 5% Sheep Blood (TSA II, Becton, Dickinson and Company, Sparks, MD) and subjected to latex agglutination test for clumping factor and protein A using the Remel BactiStaph Latex Agglutination Test (Thermo Fisher Scientific, Lenexa, KS)). Whole cell extract of each positive isolate was obtained using the Amplicor MTB Sputum Specimen Preparation Kit (Roche Molecular Systems, Inc., Indianapolis, IN) according to the manufacture's recommendations, and served as template DNA in real-time polymerase chain reactions (PCR) to amplify the *S. aureus* specific gyrA gene for organism confirmation. Oligonucleotide primers and thermal cycling conditions were used as described previously (Mertz et al. 2007), with the minor modification that 5-µl of crude lysate was used as template in initial PCR reactions instead of purified chromosomal DNA. Bacterial isolates determined to be positive for *S. aureus* specific gyrA were subjected to additional PCR to test for the methicillin resistance gene, mecA, as a marker for MRSA, and for the toxin gene pvl to evaluate the pathogenic potential of isolated organisms as previously described (Mertz et al. 2007). *Staphylococcus* cassette chromosome methicillin, SCCmec, typing was performed by the method of Oliveira and de Lencastre (2002), and Staphylococcus protein A, spa, typing was performed as described (Shopsin et al., 1999); sequences were analyzed using RIDOM spa type server for all MRSA isolates (Sinigalliano et al. 2010)

Initial sample processing for Molecular Analysis

For the preparation of total microbial community genomic DNA from each individual beach water sample, 1 liter sub-samples were aseptically filtered onto 0.45um pore-sized Whatman nitrate cellulose filters. In cases where that much volume could not be passed through the filter, the samples were filtered until clogging failure, then the actual amount of sample filtered was recorded and used in subsequent quantitation and recovery calculations. These DNA filters were then stored at minus 80°C until DNA extraction and qPCR analysis. The filters were then processed (maximum 3 weeks) for nucleic acid extraction for molecular analyses (Sinigalliano et al. 2010)

Total Genomic DNA was extracted from these filters using the FastDNA spin Kits (MPBiomedicals/Qbiogene Cat#6540-600) as per manufacturer's instructions, with the following modifications: Filters were placed in "Lysing Matrix A" bead beat tubes, and supplemented with $1x10^5$ cells from an extraction control cell suspension (*Lactococcus lactis* cells, washed 3 times in 1X PBS, then independently enumerated for whole cells counts by flow cytometry, and by direct microscopic fluorescent cell counting with both AODC and SybrGreen staining, while remaining exogenous DNA in the final washed control culture was determined by Fluorometric measurement of the cell-free supernatant using a Qubit Fluorometer with the QuantIt ds DNA quantitation Kit by Invitrogen/Molecular Probes). Filters were beaten in a Qiagen FastPrep 120 instrument for 45 seconds at a speed setting of 5.5, centrifuged at 14,000xg for 5 minutes, and the supernatant transferred to a fresh 2mL microfuge tube. The supernatant was then further purified following the Kit manufacturer's instructions, and the purified total microbial community DNA was eluted from spin columns in final100uL volumes, then aliquots were stored frozen at -80°C until further qPCR analysis (Sinigalliano et al. 2010)

Quantitative PCR Analysis of Purified DNA Extracts from Environmental Samples: DNA extracts were analyze by real-time fluorescent qPCR for general enterococci using: a) two different primers (referenced here as qPCR-a and qPCR-b) as described by Haugland et al. (2005) as entero1 and Siefring et al. (2008) as entero2, respectively; b) two human-host-specific Bacteroidales (HF8 as based on Bernhard and Field 2000a, 2000b, and Bac-Hum UCD as described by Kildare et al. 2007); c) one canine-host-specific Bacteroidales (based on Dick et al. 2005); and, d) one gull-specific *Catellicoccus marimammalium* (based on Lu et al. 2008, with Taqman probe developed as part of the current study) (Sinigalliano et al. 2010).

The primers and probe sequences used, with associated references, are shown in Table 1. All qPCR assays were run on a Chromo4 real-time qPCR instrument (BioRad/MJResearch) using the following reaction conditions: 1uL sample DNA extract (containing the spiked extraction/inhibition controls), 0.125uL each of forward and reverse primers (100uM stock), 0.1uL of Taqman probe (100uM stock), 12.5uL of commercial 2X mastermix (Qiagen QuantiTect Probe Mastermix, Cat# 204343), and 11.25uL of sterile PCR-grade water were used, giving a total reaction volume of 25uL. Cycling conditions were 15 min. denaturation at 95°C, followed by 45 cycles of 95°c for 15 sec and 60°C for 1 min with a fluorescent plate read at the end of each extension. All analyses were run in singleplex, requiring different 1 µl aliquots from the 100 µl DNA extract for each separate target assay (Sinigalliano et al. 2010)

Quantitation was determined from a serial dilution standard curve of target DNA concentrations ranging from 1 GE to 1×10^6 GE of purified DNA from *Enterococcus faecalis* (ATCC # 19433) for the entero1 assay or human-source *Bacteroides dorei* (DSM # 17855) respectively for the human-source assays of the Bacteroidales group (i.e. BacHum-UCD and Bac-HF8). The Bacteroidales – dog assay and the *Catellicoccus* – gull assay did not have genomic controls at this time, so plasmid controls containing cloned single copies of the target sequence were utilized. For the canine-host

Bacteroidales assay, control plasmids consisted of positive amplicons from dog feces DNA extract amplified with the DogBac primers (Table 1), and cloned into a plasmid vector at 1 copy per plasmid using the Zero Blunt Cloning Kit (Invitrogen) in the same manner as described below for the *Catellicoccus* control plasmid. This DogBac positive control plasmid was serially diluted in 1X TE buffer with 40ng/mL of Poly-A potassium salt (Sigma Cat# P9403-25MG) to make a standard curve ranging from 10 to $1x10^7$ plasmid target sequence copies (TSC). Thus the units of measure reported for the canine-host-specific Bacteroidales are in Target Sequence Copies (TSC) rather than in Genome Equivalence Units (GEU) as for the other qPCR assays.   For the gull-host *Catellicoccus marimammalium* assay a plasmid standard was constructed by cloning a PCR product of the *C. marimmalium* specific primers with DNA extracted from gull feces as its template. The product was run on a 1% agarose gel and the 453 bp product was excised and purified using the Qiagen gel extraction kit. The purified product was then cloned into a zero blunt TOPO plasmid using the Invitrogen Zero Blunt Cloning Kit. The plasmid was transferred into Chemi competent cells and grown on Luria Broth (LB) plus Kanamycin agar plates and colonies were selected and placed into cell-pop qPCR and also into 5 ml of LB with Kanamycin . The colonies that were positive for the target sequence were spun down and had their plasmids extracted. These extractions were performed with Promega wizard plus mini prep kit. Sample plasmid concentrations were quantified by a flourometer and copy number of the plasmid was assigned by using the plasmid and insert size (3931 bp which includes the total plasmid plus single copy insert size) (Sinigalliano et al. 2010).

The extraction/purification efficiency and potential for inhibition was measured for each sample filter extraction by the use of the known amounts of the *Lactococcus lactis* extraction control cells (Siefring et al., 2008) that were added to each sample filter and co-purified along with the sample. The percent recovery of the known *L. lactis* target was then measured for each sample by qPCR for this target with the control prmers/probe listed in Table 1. The combined recovery as a result of both inhibition and extraction efficiencies was then determined by measuring how much of the specific *L. lactis* control gDNA target sequence was left in the elution. Calculated sample quantitations were then corrected for recovery efficiency and inhibition using the measured recovery efficiencies of the *Lactococcus* controls for each sample. Recoveries below the typical range of extraction efficiences (i.e.outside 20-50%) and/or the lack of amplification of the other multiple targets from a sample flagged that sample as "inhibited" and samples demonstrating any potential inhibition were diluted and reanalyzed. Samples were not normalized for total amount of community DNA extract added to reactions, but rather used equal volumes of extract per reaction, as the samples were already being normalized for variations target sequence extraction by the extraction controls. Of note, the extraction control utilized for this particular environmental matrix was chosen such that it did not naturally occur in the environmental background of the particular beach samples being tested, and has previously been shown as an effective calibrator of extraction efficiency for both *Enterococcus* and *Lactococcus* (Siefring et al., 2008). The lack of environmental background for the calibrator signal was verified by a series of no-*Lactococcus*-spike negative control filters of beach sample water were analyzed during the course of the study by the *L. lactis* qPCR control assay to characterize any potential

Lactococcus background signal in the sample site. While it is true that *Lactococcus* controls have been problematic in some other habitats, and that this particular calibrator is not always an appropriate choice for certain environmental matrices, in this case no *Lactococcus* background was seen for any samples from the beach studied here. This particular beach has been used in a variety of qPCR studies over a period of at least 3 years and in that time, no significant environmental background *Lactococcus* qPCR signal has been observed from any of the environmental water or sand samples from it (Sinigalliano et al. 2010)

For each sampling day, one set of samples was analyzed in triplicate for all microbial measures. Results of this analysis indicated that quality control for sample processing and microbial assays were adequate for recreational water monitoring (e.g. the average percent errors for ENT(MF) was 17%).

Weather Stations

Tide and wind data were obtained from National Oceanic and Atmospheric Administration (NOAA) stations. Tide was obtained from the "Bear Cut" monitoring site located on Virginia Key, FL within 1 km from the sampling site (http://tidesandcurrents.noaa.gov/data_menu.shtml?stn=8723214%20Virginia%20Key,%20FL&type=Historic%20Tide%20Data). Wind data was obtained from the "Fowey Rocks" monitoring site located at approximately 18 km from the site (http://www.ndbc.noaa.gov/station_history.php?station=fwyf1).

Rainfall and solar radiation were measured at the Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami, which is located within 1 km from the sampling site. Rainfall was measured using 6 different tipping bucket rain gauges located within the RSMAS campus (Dr. Peter Minnett, personal communication). Solar radiation (http://www.rsmas.miami.edu/etc/download-weatherpak.cgi?file=;dir=2007) was measured using a Precision Spectral Pyranometer (PSP). The PSP measures incoming short wave radiation (W/m^2) which is a measurement of the radiative energy flux from the sky in what is loosely termed the solar spectrum. It includes the visible part of the spectrum, which is comprised of direct sunlight, scattered skylight and light scattered/reflected from clouds. It is dependent on clouds, especially if they obscure the sun.

Supplemental Table 1. qPCR primers and Taqman probes used in study (from Sinigalliano et al. 2010)

Assay	Target	Primer/Probe Sequences	Reference
Enterococci qPCR-a	General Enterococci 23S rRNA gene	Forward primer: ECST748F 5'-AGAAATTCCAAACGAACTTG-3' Reverse primer: ENC854R 5'-CAGTGCTCTACCTCCATCATT-3' Probe: GPL813TQ: 5'-6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-BHQ1-3'	Haugland et al 2005
Enterococci qPCR-b	Large subunit rRNA (139 bp)	Forward primer: Entero2 5'-GAGGACCGAACCCACGTA-3' Reverse primer: ENC854R 5'-CAGTGCTCTACCTCCATCATT-3' Probe: Entero2 5'-ACCCACACCTCATCCCCGCACTTTTC-3'	Siefring et al 2008
Bacteroidales - UCD	Human-host-specific Bacteroidales 16S rRNA gene	Forward primer: 160f 5'-TGAGTTCACATGTCCGCATGA-3' Reverse primer: BacHum-241r 5'-CGTTACCCCGCCTACTATCTAATG-3' Probe: BacHum-193p 5'-6FAM-TCCGGTAGACGATGGGGATGCGTT-BHQ1-3'	Kildare et al 2007
Bacteroidales - Dog	Canine-host-specific Bacteroides 16S rRNA gene	Forward primer:DF475F 5'-CGCTTGTATGTACCGGTACG-3' Reverse primer: Bac708R 5'-CAATCGGAGTTCTTCGTG-3' Probe: DogBac probe 5'-6FAM-ATTCGTGGTGTAGCGGTGAAATGCTTAG-BHQ1-3'	Dick et al 2005 & this study
Bacteroidales - HF8	Human-host-specific HF8 gene cluster Bacteroidales 16S rRNA gene	Forward primer: HF183F 5'-ATCATGAGTTCACATGTCCG-3' Reverse primer: Bac708R 5'-CAATCGGAGTTCTTCGTG-3' Probe: 5'-6FAM-TCCGGTAGACGATGGGGATGCGTT-BHQ1-3'	Bernhard & Field 2000a
***Catellicoccus marimammalium*- Gull**	Gull host-specific *Catellicoccus marimammalium* 16S rRNA gene	Forward primer: 5'-TGCATCGACCTAAAGTTTTGAG-3' Reverse primer: 5'-GTCAAAGAGCGAGCAGTTACTA-3' Probe: Gull2 Taqman, 5'-6FAM-CTGAGAGGGTGATCGGCCACATTGGGACT-BHQ1-3'	Lu et al 2008 Probe: this study
Lactococcus - Control	*Lactococcus lactis* whole cell extraction control 16s rRNA gene	Forward primer: 5'-GCTGAAGGTTGGTACTTGTA-3' Reverse primer: 5'-TCAGGTCGGCTATGTATCAT-3' Probe: 5'-6FAM-TGGATGAGCAGCGAACGGGTGA-BHQ-3'	Siefring et al 2008

Calculations for accuracy tests for the CS methods and regression model

$$\text{Type I error (false positive)} = b/(b+d) \quad [1]$$

$$\text{Type II error (false negative)} = c/(a+c) \quad [2]$$

$$\text{Observed agreement (OC)} = (a + d)/N \quad [3]$$

$$\text{Chance agreement (CO)} = ((a+c)/N*(a+b)/N)+((b+d)/N*(c+d)/N) \quad [4]$$

$$\text{Kappa} = (OA - CA)/(1-CA) \quad [5]$$

Where *a* is the number of samples whose enterococci results exceeded the guideline based on both conventional (MF) and alternative methods (CS and two qPCR measurements or regression models). *b* is the number of samples whose enterococci level exceeded the guideline based on the alternative methods while the MF method was within the guideline. *c* is the number of samples whose results were within the guideline based on the alternative methods while the MF method exceeded the guideline. *d* is the number of samples whose results are within the guideline based on both MF and alternative methods. N is the total number of samples used for stepwise regression. Kappa compares the agreement against that which might be expected by chance (Figure S-1).

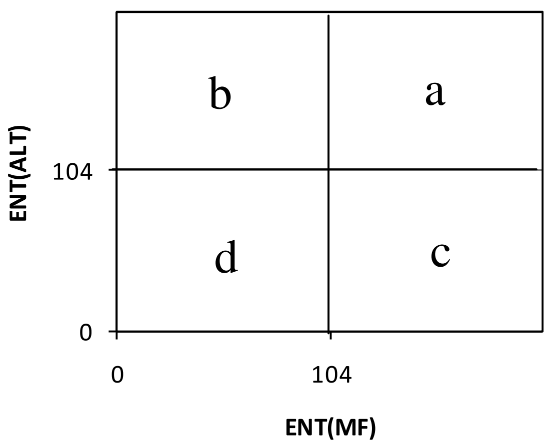

Figure S-1: Explanation of variables used in accuracy tests. ENT(ALT) corresponds to enterococci analyses by alternative methods.

Supplemental Table 2. Summary of physico-chemical parameters of individual water samples (n = 668) and hydrometrologic conditions during sampling periods

	Time	pH	Salinity (PSU)	Temp[a] (°C)	Turbidity (NTU)	Tide (m)	6hr-rain (mm)	24hr -rain (mm)	WDIR[b] (°)	WSP[c] (m/s)	Solar[d] (W/m^2)
Range	7:44 AM	6.1	32.1	20.6	0.4	0.01	0	0	0	0	3
	11:52 AM	8.8	38.6	31.3	117	0.77	12	28	357	12.8	855
Average	9:52 AM	8.0±0.03[e]	35.7±0.1	26.0±0.2	12±1.0	0.36±0.02	1±0.2	4±0.6	160±6.0	5.2±1.9	338±16

[a]Temp = temperature
[b]WDIR = wind direction
[c]WSP = wind speed
[d]Solar = solar radiation
[e]The values that follow the "±" symbol correspond to the 95% confident limits.

Supplemental Table 3. Correlation coefficient between indicator bacteria and environmental parameters. The number in the parenthesis is the “p” value.

		ENT(MF)	ENT(CS)	ENT(qPCR-a)	ENT(qPCR-b)	BAC_{UCD}	BAC_{HF8}	*S. aureus*	BAC_{dog}	CAT_{gull}
Time	IND[a]	-0.32 (<0.01)	-0.38 (<0.01)	-0.01 (0.71)	-0.02 (0.67)	-0.03 (0.41)	-0.13 (<0.01)	-0.07 (0.06)	-0.08 (0.03)	0.14 (<0.01)
	DGM[b]	-0.35 (0.20)	-0.40 (0.14)	-0.14 (0.62)	-0.08 (0.78)	-0.14 (0.63)	-0.35 (0.20)	-0.21 (0.46)	0.07 (0.81)	0.68 (0.01)
pH	IND	0.00 (0.92	0.09 (0.02)	0.04 (0.34)	0.06 (0.15)	-0.05 (0.19)	0.03 (0.48)	-0.10 (0.01)	-0.27 (<0.01)	-0.52 (<0.01)
	DGM	0.17 (0.55)	0.10 (0.74)	0.15 (0.59)	0.12 (0.66)	-0.11 (0.77)	-0.08 (0.78)	-0.21 (0.45)	-0.58 (0.02)	-0.58 (0.02)
Salinity	IND	0.05 (0.18)	-0.01 (0.88)	0.04 (0.27)	0.09 (0.02)	-0.05 (0.21)	-0.06 (0.11)	0.00 (1.00)	-0.05 (0.16)	0.16 (<0.01)
	DGM	0.28 (0.31)	0.08 (0.79)	0.31 (0.25)	0.38 (0.16)	-0.01 (0.97)	-0.48 (0.07)	-0.06 (0.83)	-0.34 (0.22)	0.07 (0.79)
Temp	IND	0.02 (0.69)	0.02 (0.62)	-0.03 (0.49)	-0.02 (0.71)	-0.27 (<0.01)	-0.03 (0.40)	0.01 (0.88)	-0.32 (<0.01)	-0.73 (<0.01)
	DGM	0.11 (0.72)	0.10 (0.74)	-0.05 (0.86)	-0.08 (0.79)	-0.41 (0.16)	0.04 (0.90)	0.12 (0.71)	-0.47 (0.11)	-0.88 (<0.01)
Turbidity	IND	0.01 (0.77)	0.16 (<0.01)	0.23 (<0.01)	0.13 (<0.01)	-0.14 (<0.01)	-0.04 (0.36)	-0.01 (0.78)	0.20 (<0.01)	0.19 (<0.01)
	DGM	0.36 (0.19)	0.46 (0.09)	0.40 (0.14)	0.25 (0.38)	-0.29 (0.30)	-0.14 (0.62)	-0.21 (0.44)	-0.11 (0.71)	0.32 (0.25)
Tide	IND	0.32 (<0.01)	0.34 (<0.01)	0.25 (<0.01)	0.24 (<0.01)	0.15 (<0.01)	0.13 (<0.01)	0.02 (0.54)	0.46 (<0.01)	0.22 (<0.01)
	DGM	0.49 (0.07)	0.58 (0.02)	0.24 (0.40)	0.28 (0.31)	0.37 (0.17)	0.46 (0.08)	-0.07 (0.81)	0.64 (0.01)	0.31 (0.26)
Rain 6hr	IND	0.12 (<0.01)	0.11 (0.01)	0.24 (<0.01)	0.17 (<0.01)	-0.14 (<0.01)	-0.06 (0.11)	-0.07 (0.05)	-0.21 (<0.01)	0.07 (0.09)
	DGM	0.46 (0.08)	0.44 (0.10)	0.39 (0.15)	0.26 (0.36)	-0.30 (0.27)	-0.21 (0.46)	-0.21 (0.46)	-0.29 (0.30)	0.22 (0.43)
Rain 24hr	IND	0.03 (0.49)	0.12 (<0.01)	0.11 (0.01)	0.06 (0.12)	-0.08 (0.05)	-0.03 (0.44)	-0.01 (0.72)	-0.15 (<0.01)	-0.24 (<0.01)
	DGM	0.12 (0.67)	0.18 (0.52)	0.22 (0.43)	0.20 (0.48)	-0.02 (0.94)	-0.20 (0.47)	-0.02 (0.94)	-0.34 (0.22)	-0.27 (0.33)
WDIR	IND	0.23 (<0.01)	0.07 (0.09)	0.12 (<0.01)	0.04 (0.29)	0.16 (<0.01)	0.08 (0.04)	-0.06 (0.11)	0.10 (0.01)	0.24 (0.01)
	DGM	0.46 (0.09)	0.20 (0.48)	0.28 (0.32)	0.20 (0.48)	0.22 (0.44)	0.04 (0.89)	-0.35 (0.20)	-0.02 (0.95)	0.31 (0.26)
WSP	IND	-0.11 (0.01)	-0.11 (<0.01)	-0.16 (<0.01)	-0.21 (<0.01)	-0.28 (<0.01)	-0.02 (0.68)	-0.03 (0.48)	-0.06 (0.12)	0.09 (0.02)
	DGM	-0.17 (0.55)	-0.02 (0.94)	-0.34 (0.22)	-0.44 (0.10)	-0.55 (0.04)	0.09 (0.75)	-0.22 (0.44)	0.13 (0.64)	0.22 (0.44)
Solar	IND	-0.22 (<0.01)	-0.32 (<0.01)	0.00 (0.90)	0.00 (0.96)	-0.03 (0.41)	-0.10 (0.01)	-0.05 (0.19)	-0.12 (<0.01)	0.03 (0.43)
	DGM	-0.16 (0.57)	-0.28 (0.31)	-0.06 (0.83)	-0.01 (0.98)	0.00 (0.99)	-0.15 (<0.01)	-0.22 (0.44)	-0.02 (0.95)	0.20 (0.47)

[a]IND = Individual Samples[b]DGM = Daily Geometric Means
[a]Temp = temperature
[b]WDIR = wind direction
[c]WSP = wind speed
[d]Solar = solar radiation

Supplemental Table 4. Summary of microbial measurements from the 668 water samples collected from this study.

		Enterococci				Human markers			Animal markers	
Parameters[b]		ENT(MF) CFU/100mL	ENT(CS) MPN/100mL	ENT(qPCR-a) GEU/100mL	ENT(qPCR-b) GEU/100mL	BAC_{UCD} GEU/100mL	BAC_{HF8} GEU/100mL	*S. aureus*[a] CFU/100mL	BAC_{dog} TSC/100mL	CAT_{gull} TSC/100mL
	Detection	86%	78%	99%	94%	58%	4%	37%	50%	67%
IND	Min	<2	<10	1	<1	<1	<1	<2	<1	<1
	Max	3,320	2,840	33,600	54,200	10,500	236	780	134,000	20,500
	AM	71±19[c]	65±15	470±172	444±172	67±36	2±1	17±5	1,100±447	924±193
	Mdn	19	30	142	145	3	<1	<2	≤1	61
DGM	Min	2	9	10	6	2	<1	1	1	1
	Max	98	88	533	434	35	<1	7	2,350	2170
	AM	27±15	36±13	181±6	161±74	7±4	<1	3±1	319±362	334±289
	Mdn	13	27	131	113	3	<1	3	9	65

[a]Values for *S. aureus* correspond to the confirmed values. 37% of the samples were confirmed positive for *S. aureus*.

[b]IND = Individual Samples, DGM = Daily Geometric Mean, AM = Arithmetic mean, Mdn = Median

[c]The values that follow the "±" symbol correspond to the 95% confident limits.

Supplement Table 5. Correlation coefficients, r, between indicator bacteria.

		ENT(CS)	ENT(qPCR-a)	ENT(qPCR-b)	BAC_{UCD}	BAC_{HF8}	*S. aureus*	BAC_{dog}	CAT_{gull}
ENT(MF)	IND[a]	0.56 (<0.01)	0.37 (<0.01)	0.37 (<0.01)	0.06 (0.13)	0.09 (0.02)	0.03 (0.52)	-0.01 (0.80)	-0.01 (0.81)
	DGM[b]	0.83 (<0.01)	0.67 (0.01)	0.71 (<0.01)	0.36 (0.19)	0.18 (0.53)	-0.10 (0.71)	-0.14 (0.62)	-0.04 (0.88)
ENT(CS)	IND		0.42 (<0.01)	0.39 (<0.01)	0.09 (<0.03)	0.08 (0.04)	-0.03 (0.40)	0.10 (0.01)	-0.06 (0.12)
	DGM		0.66 (0.01)	0.68 (0.01)	0.37 (0.18)	0.33 (0.23)	-0.11 (0.69)	0.04 (0.87)	-0.06 (0.84)
ENT(qPCR-a)	IND			0.76 (<0.01)	0.23 (<0.01)	0.04 (0.25)	0.04 (0.34)	0.07 (0.09)	0.04 (0.33)
	DGM			0.95 (<0.01)	0.38 (0.17)	0.01 (0.98)	0.26 (0.35)	-0.19 (0.50)	-0.05 (0.87)
ENT(qPCR-b)	IND				0.28 (<0.01)	0.05 (0.02)	-0.03 (0.49)	0.10 (0.01)	0.05 (0.24)
	DGM				0.45 (0.09)	-0.02 (0.94)	0.17 (0.56)	-0.19 (0.50)	-0.05 (0.86)
BAC_{UCD}	IND					0.28 (<0.01)	-0.10 (0.01)	0.27 (<0.01)	0.15 (<0.01)
	DGM					0.52 (0.05)	-0.17 (0.55)	0.28 (0.31)	0.14 (0.63)
BAC_{HF8}	IND						0.00 (0.99)	0.25 (<0.01)	0.02 (0.60)
	DGM						-0.07 (0.81)	0.47 (0.08)	-0.17 (0.54)
S. aureus	IND							-0.01 (0.87)	-0.03 (0.39)
	DGM							0.04 (0.89)	-0.28 (0.31)
BAC_{dog}	IND								0.34 (<0.01)
	DGM								0.42 (0.12)

The number within parenthesis is “p” value.

[a]IND = Individual Samples

[b]DGM = Daily Geometric Means

Supplemental Table 6. Storm event sample results including results from two samples (A and B) of runoff water collected from natural drainage ditches, two samples of water collected in ankle deep water and two samples of water collected from knee deep water. Ankle and knee deep water samples were located immediately downstream from the runoff ditches.

		Enterococci				Human markers			Animal markers	
		ENT(MF) CFU/100mL	ENT(CS) MPN/100mL	ENT(qPCR-a) GEU/100mL	ENT(qPCR-b) GEU/100mL	BAC_{UCD} GEU/100mL	BAC_{HF8} GEU/100mL	*S. aureus*[a] CFU/100mL	BAC_{dog} TSC/100mL	CAT_{gull} TSC/100mL
Runoff	A	25,200	17,300	23,500	81,100	<1	<1	< 2	50	126,000
	B	115,000	>48,400	130,000	347,000	<1	<1	< 2	<1	213,000
	AM	70,100	n/a	77,000	214,000	<1	<1	< 2	25	170,000
Ankle	A	8,370	10,400	4,530	29,600	<1	<1	< 2	257	8,740
	B	7,310	12,200	3,650	22,700	<1	<1	< 2	379	512
	AM	7,840	11,300	4,090	26,100	<1	<1	< 2	318	4,630
Knee	A	2,080	2,600	3,530	76,700	<1	<1	< 2	75	4,000
	B	833	2,330	203	1,200	<1	<1	< 2	262	3,010
	AM	1,460	2,460	1,860	38,900	<1	<1	< 2	168	3,510
Individual	After rain[b]									
	AM	3,000	2,590	8,142	151	2	<1	7	<1	3,710
	±	387	373	4390	96	2	<1	12	<1	510
	No rain[c]									
	AM	151	95	335	371	360	14	8	1,690	415
	±	101	38	183	219	530	16	7.1	900	295

[a]Values for *S. aureus* correspond to the confirmed values.
[b]Arithmetic mean (AM) of the first three individual samples collected after rainfall stopped on March 8 and June 13 (n=3).
[c]Arithmetic mean of the first three individual samples collected on non-rain days during the monitoring periods (n = 36). ± indicates 95% confidence limits. The reason that the first 3 individual samples were averaged was to control for variations in solar radiation. The solar radiation during the first three samples for each sample day were much closer to one another (75±48) in comparison to the solar radiation measures for the entire sampling period, as solar radiation tended to increase throughout the course of sampling from early morning to early afternoon.

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